REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
29-11-2012	Final Report	15-May-2009 - 14-May-2012

4. TITLE AND SUBTITLE
Final Report on Controlled Assembly of Rod-Like Particles

5a. CONTRACT NUMBER
W911NF-09-1-0236

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS
Qian Wang

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of South Carolina Research Foundatio
South Carolina Research Foundation
901 Sumter ST, 5th floor Byrnes
Columbia, SC 29208 -0001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
56056-CH.12

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
The generation of nano materials with hierarchical ordered structure is the basis for the development of novel optical, electronic, acoustic and magnetic materials. Plant viruses can be considered as nature nanoparticles that can be tailored chemically and genetically. Compared with the inorganic nanoparticles, the uniform shape and size of viruses provide highly promising possibilities in self-assembly study for the construction of nanoscale materials with hierarchical ordering. Mutagenesis of tobacco mosaic virus has been well documented for its interactions with

15. SUBJECT TERMS
self-assembly; virus; rod-like particles; tobacco mosaic virus; biomaterials

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Qian Wang
UU	UU	UU	UU		19b. TELEPHONE NUMBER 803-777-8436

Report Title

Final Report on Controlled Assembly of Rod-Like Particles

ABSTRACT

The generation of nano materials with hierarchical ordered structure is the basis for the development of novel optical, electronic, acoustic and magnetic materials. Plant viruses can be considered as nature nanoparticles that can be tailored chemically and genetically. Compared with the inorganic nanoparticles, the uniform shape and size of viruses provide highly promising possibilities in self-assembly study for the construction of nanoscale materials with hierarchical ordering. Mutagenesis of tobacco mosaic virus has been well documented for its interactions with other viral proteins during viral gene replication, movement across plant tissues, and assembly/reassembly processes. In this project, on the basis of the surface modification of plant viruses with chemical and genetic methods, we can control the self-assembly of spherical viral particles and rod-like tobacco mosaic virus to form 1D, 2D and 3D self-assemblies. Synchrotron-based small angle neutron scattering and x-ray scattering offer us powerful methods to quantitatively analyze these assembled structures.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
10/20/2011	2.00	Zhaohui Su, Qian Wang. A Hierarchical Assembly Process to Engineer a Hydrophobic Core for Virus-like Particles, Angewandte Chemie International Edition, (12 2010): 0. doi: 10.1002/anie.201005548
10/20/2011	1.00	Jianhua Rong, Zhongwei Niu, L. Andrew Lee, Qian Wang. Self-assembly of viral particles, Current Opinion in Colloid & Interface Science, (9 2011): 0. doi: 10.1016/j.cocis.2011.09.001
10/20/2011	3.00	Yuan Lin, Zhaohui Su, Guihua Xiao, Elizabeth Balizan, Gagandeep Kaur, Zhongwei Niu, Qian Wang. Self-Assembly of Virus Particles on Flat Surfaces via Controlled Evaporation, Langmuir, (02 2011): 0. doi: 10.1021/la103917x
10/20/2011	4.00	L. Andrew Lee, Huong Giang Nguyen, Qian Wang. Altering the landscape of viruses and bionanoparticles, Organic & Biomolecular Chemistry, (09 2011): 0. doi: 10.1039/c1ob05700f
10/20/2011	5.00	Qingbing Zeng, Sharmistha Saha, L. Andrew Lee, Hannah Barnhill, Jerry Oxsher, Theo Dreher, Qian Wang. Chemoselective Modification of Turnip Yellow Mosaic Virus by Cu(I) Catalyzed Azide?Alkyne 1,3-Dipolar Cycloaddition Reaction and Its Application in Cell Binding, Bioconjugate Chemistry, (01 2011): 0. doi: 10.1021/bc100351n
10/20/2011	6.00	Laying Wu, L. Andrew Lee, Zhongwei Niu, Soumitra Ghoshroy, Qian Wang. Visualizing Cell Extracellular Matrix (ECM) Deposited by Cells Cultured on Aligned Bacteriophage M13 Thin Films, Langmuir, (07 2011): 0. doi: 10.1021/la201580v
10/20/2011	7.00	Laying Wu, Jianfeng Zang, L. Andrew Lee, Zhongwei Niu, Gary C. Horvatha, Vaughn Braxtona, Arief Cahyo Wibowo, Michael A. Bruckman, Soumitra Ghoshroy, Hans-Conrad zur Loye, Xiaodong Li, Qian Wang. Electrospinning fabrication, structural and mechanical characterization of rod-like virus-based composite nanofibers, Journal of Materials Chemistry, (06 2011): 0. doi: 10.1039/c1jm00078k
10/20/2011	8.00	Sumit Kewalramani, Suntao Wang, Yuan Lin, Huong Giang Nguyen, Qian Wang, Masafumi Fukuto, Lin Yang. Systematic approach to electrostatically induced 2D crystallization of nanoparticles at liquid interfaces, Soft Matter, (10 2011): 0. doi: 10.1039/c0sm00956c
11/01/2012	9.00	Nisaraporn Suthiwangcharoen, Tao Li, Kai Li, Preston Thompson, Shaojin You, Qian Wang. M13 bacteriophage-polymer nanoassemblies as drug delivery vehicles, Nano Research, (02 2011): 483. doi: 10.1007/s12274-011-0104-2
11/01/2012	10.00	Suntao Wang, Masafumi Fukuto, Antonio Checco, Zhongwei Niu, Qian Wang, Lin Yang. Role of electrostatic interactions in two-dimensional self-assembly of tobacco mosaic viruses on cationic lipid monolayers, Journal of Colloid and Interface Science, (06 2011): 0. doi: 10.1016/j.jcis.2011.03.048
11/01/2012	11.00	Laying Wu, Tao Li, Douglas Blom, Jibin Zhao, Soumitra Ghoshroy, Qian Wang. Synthesis and electron microscopic analysis of the self-assembly of polymer and ferritin core-shell structures, Microscopy Research and Technique, (07 2011): 0. doi: 10.1002/jemt.20891
TOTAL:	**11**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper

TOTAL:

Number of Manuscripts:

Books

Received	Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Qian Wang, USC Rising Star Award, 2010
Qian Wang, NSF American Competitiveness Fellow Award, 2009
Qian Wang, South Carolina Governor's Young Scientist Award, 2009

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Jittima Luckanagul	1.00	
FTE Equivalent:	**1.00**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Andrew Lee	0.80
FTE Equivalent:	**0.80**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Qian Wang	0.05	
FTE Equivalent:	**0.05**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Quyen Nguyen	0.50	
FTE Equivalent:	**0.50**	
Total Number:	**1**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	1.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:	1.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:	1.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	1.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	1.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	1.00

Names of Personnel receiving masters degrees

NAME	
Jerry Oxsher	
Total Number:	**1**

Names of personnel receiving PHDs

NAME	
Eve Suthiwangcharoen	
Elisabeth Balizan	
Total Number:	**2**

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment

Technology Transfer

Scientific progress and accomplishments

(1) Using GISAXS study the structural diversity and anisotropy effects in 2D virus array

In the past year, we continued that investigation of two-dimensional (2D) assembly of the icosahedral turnip yellow mosaic virus (TYMV) on cationic lipid monolayers at the air-water interface. In situ x-ray scattering reveals two close-packed 2D crystalline phases of TYMV that are distinct from the previously reported hexagonal and centered square (p2 × p2) lattices. One of the observed phases arises from either a dimeric double-square (2×1) or tetrameric square (2×2) unit cell. The other is a rhombic crystal in which TYMV's 2-fold axis is oriented along the interface normal and the lattice axes are related to the particles' equatorial 3- and 5-fold axes. TYMV's anisotropy attributes suggest that the rhombic crystal arises predominantly from local hydrophobic interactions at interparticle contacts while the contacts in the (p2 × p2) crystal are stabilized by steric and electrostatic complementarity.

This study illuminates the interplay between particle anisotropy and interactions in generating long-range order. We have discovered two 2D crystal phases of TYMV that are distinct from the previously observed hexagonal and square arrays of TYMV. The existence of these multiple 2D packing motifs demonstrates the versatility of highly symmetric NPs in constructing nanostructures, even as single-component systems. Local hydrophobic interactions are identified as the dominant in-plane interactions in the rhombic crystal, whereas steric and electrostatic complementarity appears important in the square crystal. While the critical role of these interactions in protein associations is well established, our results illustrate how they may be exploited to dictate the order that emerges in many-body assemblies.

(2) Incorporation of TMV into alginate hydrogels

As shown in ***Figure 1***, we recently developed composite materials using porous alginate hydrogel (PAH) and TMV particles for the purpose of cell culturing studies. The porous alginate hydrogel fabrication followed the protocol reported by Barbetta et al, and TMV particles were introduced by physically mixing TMV particles during alginate hydrogel synthesis. To verify this simple incorporation method and to confirm that the incorporated virus still maintains its original structure, the existence of TMV intact particles in the hydrogels after the entire synthesis process was observed under TEM after de-crosslinking of alginate hydrogel. The virus-incorporated alginate hydrogel was also shown to have similar physical properties including swelling property, stability, and structural basis of pore architecture. The thermal analysis (TGA and DSC) showed that incorporation of TMV did not significantly impact the thermo-stability and heat flow profile of original PAH. The amount of TMV released from TMV-alginate hydrogel into the aqueous solution was measured and it confirmed that most TMV particles were entrapped in the hydrogel matrices even upon long term incubation in solution. The incorporation of TMV in alginate hydrogel did not at all impair the pore formation or the pore architecture. The mechanical measurements indicated an increase in stiffness, indicated by incremental modulus at low strain range, was observed in virus-incorporated porous hydrogels.

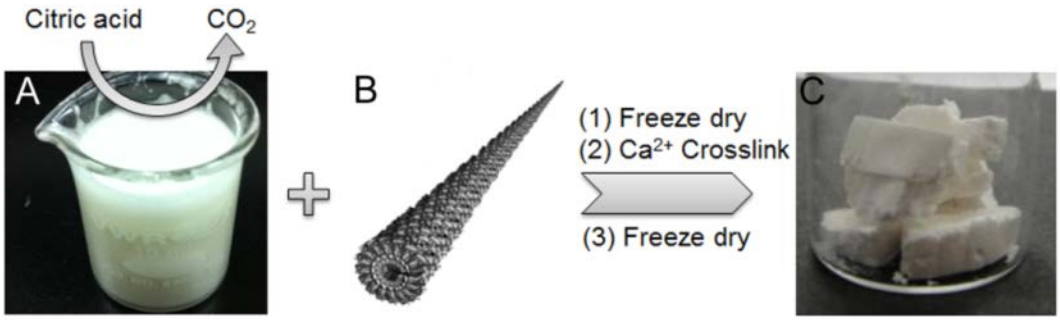

Figure 1. Synthetic procedure to generate virus functionalized porous composite hydrogels. (***A***) Alginate mixture comprised of low viscosity alginate, Pluronic F108, sodium bicarbonate with an equivalent amount of citric acid to generate gas templated foamy mixture. (***B***) TMV was added 5 min before the foamy mixture was frozen and lyophilized. The lyophilized sample was crosslinked with $CaCl_2$. (***C***) Porous composite hydrogel was obtained after dialysis against 0.1 M $CaCl_2$ and lyophilization.

(3) Formation of TMV superlattice structures in methylcellulose hydrogels

A key discovery in our laboratory, in collaboration with Drs. **Byeongdu Lee** and **Tao Li** from ANL, was that TMV can form superlattice structures when mixed in a methylcellulose hydrogel. Methylcellulose (MC, ***Figure 2***), a water soluble polymer derived from cellulose, is a temperature responsive depletant that would trigger assembly or disassembly behavior of colloidal particles mixed with it. Without MC, TMV solution is clear and only shows its form factor scattering in SAXS profile without any peaks (data not shown). Upon addition of MC, the sample becomes opaque (inset of ***Figure 2***) and the microscope image suggests that TMVs form bundle structures with several tens of micrometers in length (***Figure 2c***) while pure MC does not form any structures. ***Figure 2d*** is the 2D scattering image of the MC/TMV sample, revealing a highly ordered structure. Furthermore, the azimuthally averaged 1D SAXS profile (***Figure 2e***) shows seven sharp diffraction peaks whose positions with respect to the principal peak, $(q/q^*)^2$, are 1, 3, 4, 7, 12, 13, and 16 (where q* is the position of the principal (100) peak), which is in good agreement with the ideal diffraction pattern from a 2D hexagonal lattice. The domain size of the hexagonal bundle at 25^{o}C is around 150 nm as calculated from the full width at half maximum of the principal peak. The SAXS data clearly demonstrated the formation of superlattice structure of TMV in the MC matrix, which is further confirmed by the TEM analysis (***Figure 2f, g***).

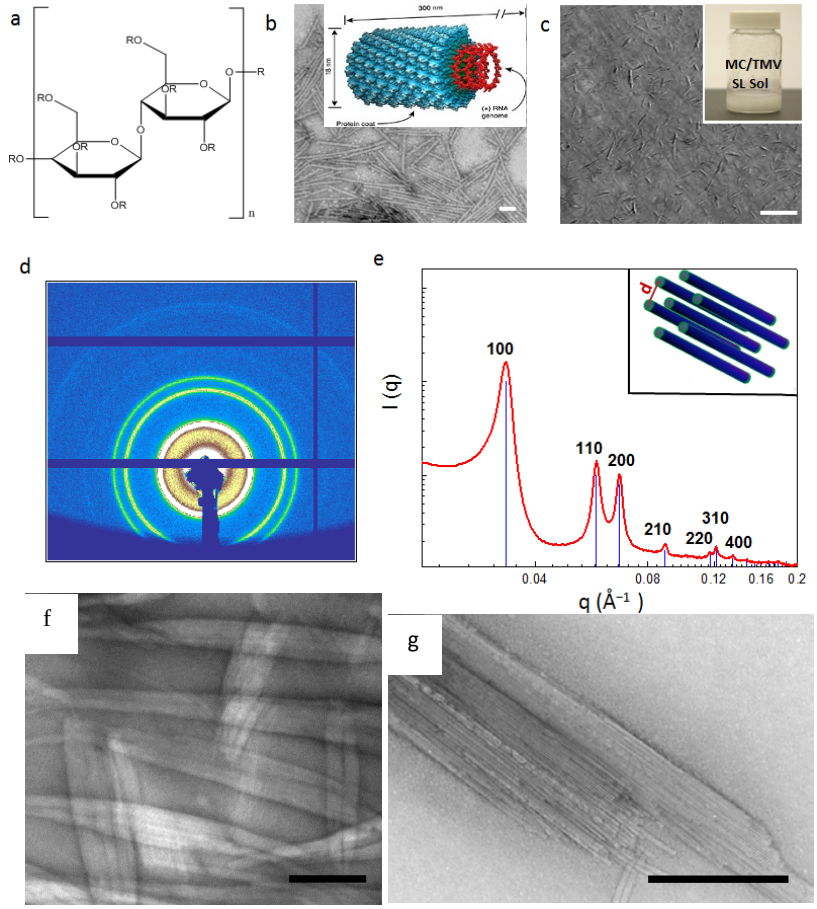

Figure 2. (***a***) Chemical structure of methylcellulose (MC). R is H or CH_3. (***b***) Transmission electron microscopy (TEM) image of TMV. Inset is the schematic illustration of the structure of TMV based on the crystal structure of TMV (mrsec.wisc.edu). (***c***) Optical microscopy image of solution of MC/TMV sample, containing 4 wt % MC in 30 mg.mL^{-1} TMV solution. Inset is its digital image. (***d***) A typical 2D SAXS image from a MC/TMV superlattice. (***e***) Indexing the 1D curve obtained by azimuthally averaging the image in (***a***) with a 2D hexagonal lattice, as shown inset of (***e***). The center-to-center spacing, or inter-TMV distance (***d***), is calculated using $d = 4\pi/\sqrt{3}q^*$. For this sample, q* is around 0.03356 Å^{-1}, which gives d = 21.6 nm. (***f, g***) TEM analysis of MC/TMV superlattice by collecting a thin-slice of the hydrogel. (***g***) is a magnified image, showing clear packing of TMV particles. Scale bars: (***b***), 100 nm; (***c***), 50 µm; and (***f & g***), 500 nm.

(4) Tobacco Mosaic Virus as a New Carrier for Tumor Associated Carbohydrate Antigen

We continue the conjugation work of tobacco mosaic virus (TMV) to display tumor associated carbohydrate antigens (TACAs). For TACAs, one serious challenge was the low immunogenecity of these antigens. In collaboration with Prof. **Xuefei Huang** (Michigan State Univ.), we employed the wild type TMV as well as the cysteine-inserted TMV as carrier to present a very weak TACA, the monomeric Tn antigen. The copper catalyzed azide-alkyne

cycloaddition reaction and thiol-maleimide reaction were used to efficiently link Tn motifs onto the TMV capsid without resorting to large excess of the Tn antigen (***Figure 3***).

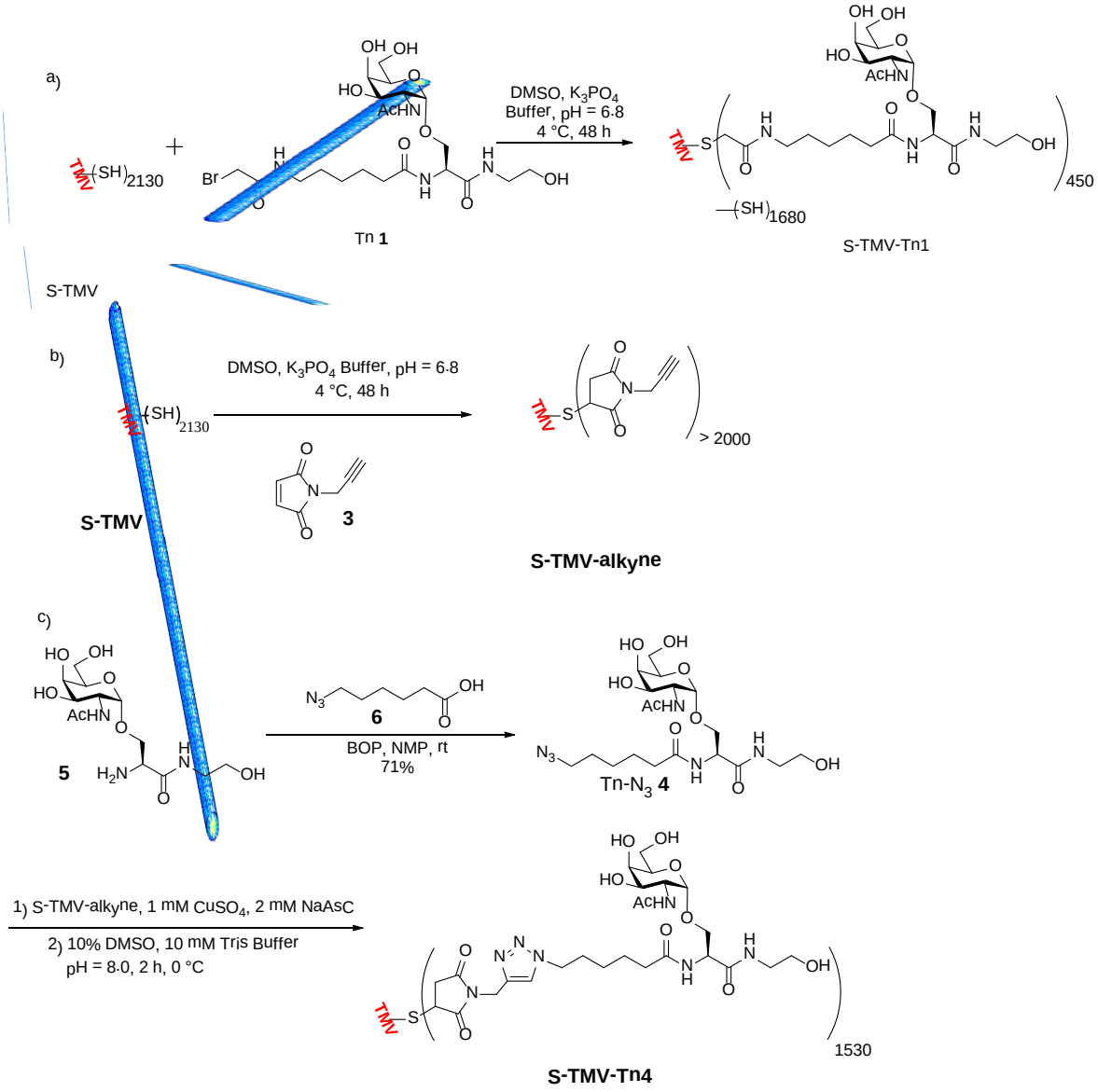

Figure 3. Conjugation of Tn to cysteine-mutated TMV (TMV1Cys).

We found that the location where Tn antigen was attached was crucial. Tn introduced at the N-terminal of TMV was immunosilent, while that attached to tyrosine 139 elicited strong immune responses. Both Tn specific IgG and IgM antibodies were generated as determined by enzyme linked immunosorbent assay and a glyco microarray screening study. The production of high titers of IgG antibodies suggested that the TMV platform contained the requisite epitopes for helper T cells and was able to induce antibody isotype switching. The antibodies exhibited strong reactivities towards Tn antigen displayed in its native environment, i.e., cancer cell surface, thus highlighting the potential of TMV as a promising TACA carrier.

(5) Collaboration with NSRDEC

Dr. Ramanathan Nagarajan (NAGU) of the Molecular Sciences and Engineering Team at NSRDEC is interested in our assembly work. He just recruited a graduate student, Dr. Eve Suthiwangcharoen to join his research team. We are working on a joint project which is seeking funding support from DTRA now.